A PHYSIOLOGICAL STUDY OF THE GERMINATION OF AVENA FATUA

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(WITH THIRTEEN FIGURES)

I. Introductory

The apparent inability of many seeds to germinate for some time subsequent to harvest has been the object of study both in Europe and in America. The vitalistic viewpoint, which early ascribed such phenomena to the inherent properties of protoplasm, obscured the whole situation. Such germinative delays were found capable of modification by external influences which were said to exert a stimulus on the seed. The later physiological studies have attempted to uncover the nature of the modifications in function which the so-called stimuli bring about.

Many seeds with low germinative power after harvest acquire increased viability in succeeding weeks or months, during a period which has been termed the after-ripening or germ-ripening period. The changes taking place during this period have been found, in general, due to alterations in the structures inclosing the seed, or to modifications in the inclosed members themselves. The term after-ripening has been limited to changes of the latter type (22), but in this paper will be used to cover all changes in the seed subsequent to harvest, as a result of which greater germination percentages may be obtained.

The most extensive investigations of germination characters among the Gramineae have probably centered on barley, an early and prompt germination of which is much sought in the malting industries. In agriculture much trouble is occasioned in the small grain regions by the persistence of the wild oat, *Avena fatua* L., and by the difficulty with which it is eliminated. As a cattle food it is undesirable because of the long and twisted awns, and also because of its lightness, averaging about 12–18 pounds per bushel. Laboratory and field tests have shown the seed to germinate very

poorly and unevenly after harvest, and later to increase in viability during succeeding weeks. In the work here described, an attempt has been made to determine the factor or factors restricting the germination of the wild oat, and to investigate the nature of the after-ripening changes taking place in the seed subsequent to harvesting.

II. Historical

1. Germination studies of the Gramineae.—It is supposed that the common cultivated oat, *Avena sativa*, has been derived from *A. fatua*. Trabut (64) holds that *A. sterilis* and *A. fatua* have given rise to two series of cultivated oats: *A. sterilis* to those of the Mediterranean region, and *A. fatua* to those of Central Europe. Instances are reported for *A. sativa* of what are thought to be reversions toward the parent stock (52, 67). The wild oat is specially abundant in the Pacific Coast region, where four varieties have been reported (20): *A. fatua*, the true wild oat; *A. fatua glabrescens*, the bastard oat; *A. barbata*, the slender oat; and *A. sterilis*, the fly oat. The wild oat has been found as far east as Illinois, but is not troublesome east of the Mississippi River. It is especially abundant in the northern small grain belt. Recently Criddle (16) has been investigating the false wild oats of Canada, which he believes represent some form of deviation from type which affects the seed coat only and leaves the seed unaltered. By the word "type" reference is made to the normal tame oats from which the false wild oats are supposed to have been derived. Yet he finds one of the most conclusive evidences, as to its distinction from *A. fatua* proper, in the greater ease with which the false wild oat may be germinated. These false wild oats resemble certain tame varieties, and may be due to crossing with *A. fatua*, although much uncertainty has been expressed as to the common occurrence of cross-pollination between *A. fatua* and *A. sativa* (67). In 1900 Kinzel (42) called attention to the progressive rise in germination percentage of *A. sativa* in the months subsequent to harvest. Atterberg (3), in his studies of the after-ripening of grain at the Swedish station, finds he can notably raise the after-ripening rate by drying the seed at higher temperatures. Wounding has also

hastened the germination of barley. He believes the temperature at which small grain will germinate is a measure of the degree of after-ripening. Thus, if seed will not grow at 13–15° C., preliminary drying is necessary; while if the seeds are capable of growing at 30° the process of after-ripening is complete. He believes the after-ripening is but the completion of the ripening processes in seeds which have been harvested so late that normal ripening has not occurred. ZADE (67) notes the delay of germination of freshly harvested *A. fatua*, and that it may be influenced by various external factors, but does not attempt to explain the effects of these various factors. He finds the delay is much shorter in seed kept in dry air of the laboratory than in that lying in the field, due as he believes to the difference in temperature. JANSON (38) notes two degrees of maturity in barley and oats, which he calls "yellow-ripeness" and "dead-ripeness." The ripening of oats up to the yellow stage results in a larger increase in the protein stuffs. After yellow-ripeness one-third of the total increment occurs, and is only nitrogen-free stuffs. He thus believes too early cutting results in considerable carbohydrate loss to oats, for the effect of after-ripening extends only to the early stages of normal ripening, which concerns proteins chiefly. KIESSLING (41) recognizes the beneficial effects of drying upon germination, but believes that germ-ripening or after-ripening is a process in general independent of seed-moisture content. He reports an elaborate series of tests under various conditions modifying germination, and concludes as regards oats that germ-ripening is a characteristic varying with the strain tried. He discredits explanations of after-ripening or germinative variations which are based on seed coat exclusions of water or gases, or on enzymatic alterations as so far set forth; yet believes that in some way enzymes are associated with germ-ripening.

2. GENERAL GERMINATION STUDIES.—No attempt will be made to cite all the recent literature on the general field of germination. However, a few instances may be cited to show that such problems have been approached from at least three different angles: (1) studies of germination factors external to the embryo; (2) factors associated with chemical or physical alterations of members within

the seed coat; (3) enzyme studies. In the studies of factors external to the embryo, CROCKER (17, 18) found the seed coat obstructs in various cases the entry of oxygen or water. A. J. BROWN in a study of *Hordeum* (9, 10) found that non-living structures inclosing the embryo have the power of excluding various salts and acids in solution. This work was extended by SCHRÖDER (59) for wheat; while REICHARD (57) would explain this peculiarity for barley by saying that there is a causal relation between the solubility of the tannins in the seed coat by various agents and the ability of these agents to penetrate the seed. Wounding and its effect on germination has been noted by various investigators (12, 17, 30, 31, 39, 41, 65). The germination rate of *A. fatua* has been increased by alternations of moisture and dryness (51). Other factors concerned with germination of seeds as so far studied are drying (3, 41, 67), freezing and thawing (23, 53, 54), effect of burying in soil (4, 21), subjection to various gases during storage and germination (41), effects of light (25–28, 35, 36, 43–47), of treatment in hot water (5, 41), and in dilute acids (22, 24).

Chemical and physical studies have been made of alterations in the endosperm or embryo of various seeds. H. T. BROWN (13) believes that in after-ripening of barley cytase, acting on the middle lamellae of the cell walls, changes the earlier glazed condition of the endosperm to a mealy structure, following which better germination is possible. JOHANNSEN (39) correlates after-ripening with fluctuations in the sugars and amide nitrogen stuffs, while ZALESKI (68) has followed the ripening process of peas and found protein increases at the expense of amino acids, amides, and organic bases. KIESSLING cites work (41) emphasizing the relation between the protein content in grasses and the speed of germination.

The third type of germination studies, dealing with enzymatic relations, includes a large number of researches. BROWN and MORRIS (12) in their study of *Hordeum* believe that the development of amylo-hydrolytic enzymes is located in the epithelial cells of the scutellum, and think the zymogens of the resting seed on germination are activated by the development of acidity. In this view as to the function of acidity in relation to enzymes, they follow the ideas of GREEN (29). In 1892 HOTTER (37) expressed

the view that the diastatic content of wheat increased in the resting seed, coming to its full possibilities at the time of germination. This after-ripening could be hastened by warming and air-drying of the seeds. DETMER (19) showed that oxygen entry is necessary for the formation of diastase. LEHMANN and OTTENWÄLDER (49) would explain the forcing of germination by various factors as due to hydrolysis of the proteins. These views are based on experiments in which temperature variations were employed, nutrient solutions were used, and also splitting products of the proteins as asparagin. ABDERHALDEN and DAMMHAHN (1) believe that germinated and ungerminated seeds vary in the presence or absence of peptolytic ferments, while APPLEMAN (2) for the potato and Miss ECKERSON (22) for *Crataegus* trace enzymatic alterations in after-ripening. In the latter case there are also found alterations in the acidity and water-holding power of the embryo. Recently, PUGLIESE (55) has investigated the autolysis of oat seeds which had not germinated, and concludes that ungerminated ones may be distinguished from the germinated ones by the presence or absence of amidases or enzymes caring for the end products of the proteolytic digestion. If such enzymatic differences exist between seeds which have been germinated and those which have not, there is quite possibly a very worthy field for investigation in the enzymatic differences between the freshly harvested and the after-ripened seeds, although these differences may not be associated with the problem of dormancy.

It is evident that the conflicts of opinion and the apparent conflicts of fact must be harmonized, if at all, only in the light of careful quantitative determinations of the various factors involved, under standard conditions, and with uniformity of experimental material.

III. Experimental

The work described in this paper has been carried on with seeds of *Avena fatua* received from the Dominion Experimental Farm at Indian Head, Saskatchewan, of the crops of 1910, 1911, and 1912. Parallel tests have been made with seed from Grand Forks, North Dakota, from Brandon, Manitoba, and with seed raised in Chicago

in the summers of 1912 and 1913.[1]   Comparisons have been made in many cases with the varieties of *A. sativa* known as Lincoln, Swedish select, Kherson, and White Tartar.

1. GERMINATION TESTS.—Germinative tests made through a considerable period showed for the wild oat seed tested the same progressive increase in percentage of germination which has been noted by other investigators for various grasses.   From many tests made those given in table I are typical, A and B representing two varieties.[2]

TABLE I

WILD OATS HARVEST OF 1911

Tested	Shell coats on		Shell coats off		In soil	
	A	B	A	B	A	B
December.........	0.5	4.3	48.0	35	....	....
January..........	....	....	70.9	....	64	....
March............	29.0	....	90.0	....	94	....
April.............	....	52.0	....	84	....	77
June.............	60.0	26.0	81.0	86	....	....
August...........	37.0	30.0	93.9	79	96	63

Germination tests were made in Petri dishes on moist absorbent cotton at a temperature of about 20° C.   Soil tests were made in the greenhouse, even watering being accomplished by the porous clay cup method (34).   As pointed out by ZADE, the germination is much better with the shell coats removed.   However, there is a rise in germination rate apparent through the after-ripening period by seeds from which they have been removed.   The peculiarities of germination are thus not to be attributed to low vitality of the seed used, nor to exclusion effects of the shell coats.

In meeting the problem of after-ripening, it is evident that changes must take place during this period either in the structures inclosing the embryo or in the inclosed members themselves. Tests were undertaken designed to furnish data on this situation. The seed coat of *Avena fatua* is formed by the combined cell layer

[1] For these samples I am indebted to Dr. M. A. BRANNON of Grand Forks, Mr. ANGUS MACKAY of Indian Head, and the Dominion Farm at Brandon.

[2] The term "shell coat" is used in this paper to include the palea and lemma inclosing the seed as opposed to the true seed coat.

remnants of the original ovary wall, the integuments, and the epidermis of the nucellus (**14, 32**). This coat cannot be removed from the endosperm and embryo without injury, and thus attention must be called to the difficulties which are immediately encountered in studying the coat effects of the oat. To know absolutely what the coat effects are is impossible; for to break the coat involves dangers of "wound effects," or of infection; while if the seeds are subjected to fluids or gases, it cannot be said with certainty that any results obtained are due primarily to the external
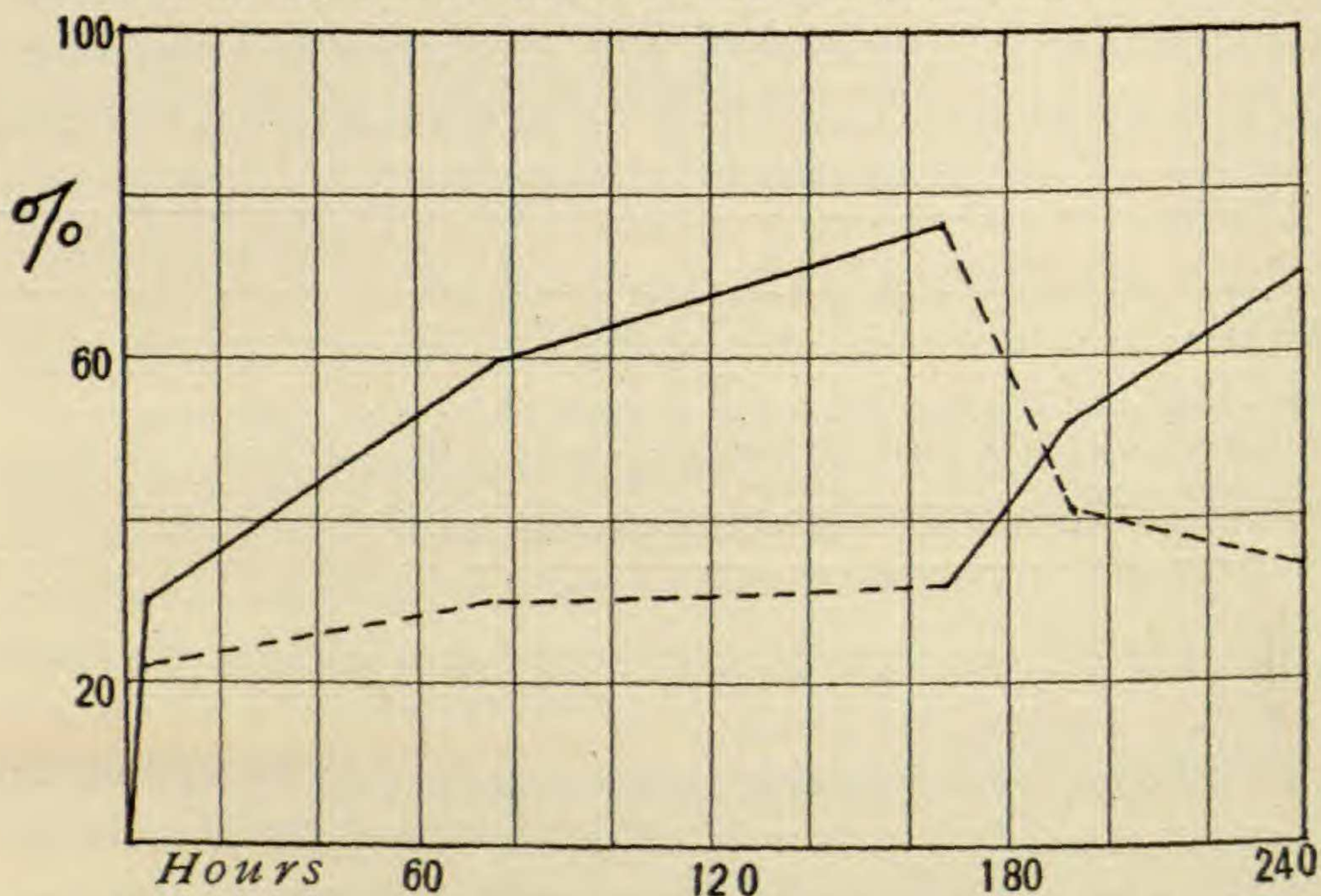
FIG. 1.—Water intake, showing semipermeability of the wild oat; increments in percentage of air dry weight; in water, solid line; in gram-molecular sodium chloride solution, broken line.

agents used, as questions of their actual entry to the embryo arise, and also the question as to what exact physiological function is set in action by such agents, even if they are successful in forcing germination. These difficulties are of course obviated in experimentation with seeds which, like *Xanthium* (**60**), permit of an easy removal of the seed coat. However, in a study of *Avena* it is necessary to gather as much data as possible from a variety of external factors, and, recognizing the uncertainty to which any one line of inquiry alone leads, to judge the situation from the combined results.

2. WATER INTAKE.—BROWN'S work on the semipermeability of *Hordeum* led to like tests of *A. fatua*. Distilled water and gram-molecular solutions of sodium chloride display on test a rather close approximation to perfect semipermeability (fig. 1). Later work by SHULL (61) in this laboratory shows that the power of excluding various salts in solution by non-living membranes is a rather common property of seed coats. It was thought possible that there might be some correlation between this behavior of *Avena* and gaseous or water exclusions in unafter-ripened seeds.
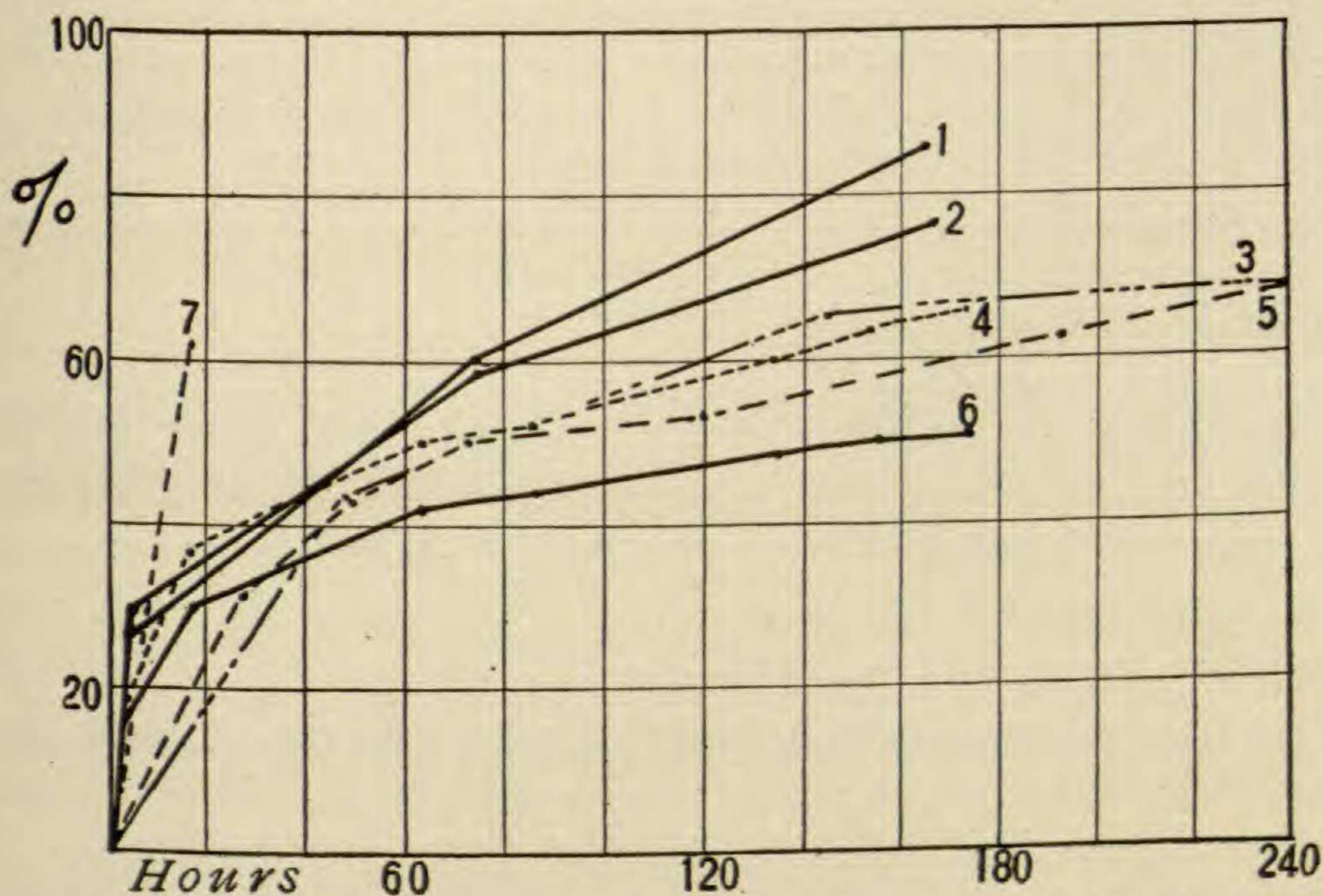

FIG. 2.—Comparative water intake among the Gramineae and in *Xanthium;* increments in percentage of air dry weight; curves: *1*, wild oats 1911; *2*, wild oats 1910; *3*, barley; *4*, *Avena sativa;* *5*, wheat; *6*, wild oats 1911; *7*, *Xanthium*.

Tests were thus made of the rate of water intake for seed of different crops and for *A. sativa*, the shell coats being removed in all cases (fig. 2). Comparing the data derived with figures given by SCHRÖDER for *Triticum* and by A. J. BROWN for *Hordeum*, there is seen to be a general similarity in the rate and total water intake for these grasses. Although much slower than the water intake of some other seeds, as for instance *Xanthium* (17), there does not seem to be any ground for saying that water exclusion in the case of *A. fatua* can explain its peculiarities of germination in the light of the general behavior of *A. sativa, Triticum,* and *Hordeum*.

3. WOUNDING.—Various attempts were then made to see if the slowness of germination soon after harvest could be influenced by other external factors. Tests of light and darkness did not seem to indicate for the wild oat the importance ascribed to these factors by German investigators for other seeds. Using a sterilized needle and pricking the true seed coat of seeds from which the shell coats had been removed tended to raise the germination percentage as pointed out by several other workers. The results given in table II were typical.

TABLE II

No. tested	Month	Per cent germination	
		Pricked	Check
100..............................	December 1911	100	35
100..............................	December 1912	97	48

As regards the relation of seed wounds and water intake, COUPIN (15) has shown that wounds increase the speed of intake, but have no influence on the maximum absorbing power. In order to accomplish the same result of breaking the seed coat without so much danger of infection, the method was adopted of searing the seed near the embryo with a red hot needle. Similar increases in germinative percentage were again noticed. To determine whether the modified behavior of seared seeds could be attributed to "wound effects" of a temporary character, dry seeds were seared, and after a month compared with other seeds previously soaked in water for 24 hours in the ice chest and then seared just before placing in the germinator. No marked difference could be noted in the two lots. Typical results of searing the seeds of *A. fatua* are shown in table III.

TABLE III

Time	No. seeds	Per cent germination	
		Seared	Check
February 1912.................	100	95	74
March 1912...................	100	100	90
December 1912...............	100	99	52
December 1912...............	100	95	64
January 1913.................	100	100	67
January 1913.................	100	98	68

It will be noticed that the difference between the checks and the treated seeds becomes increasingly less conspicuous with the passage of time after harvest, that is, as after-ripening progresses. Tests were made of fresh seed hardly yet "out of the milk," which tend to show that the seed is then neither lacking in vitality nor under the necessity of a long process of drying to secure "necessary protoplasmic alterations." One test (table IV) will show the tendency.

TABLE IV

No. tested	Period	Per cent germination	Then	Additional percentage	Total percentage
50	4 days	28	Seared 3 days.............	70	98
50	4 "	36	In 40 per cent O_2 3 days.....	34	70

In this connection it is interesting to note that ZADE found that seed harvested unripe yielded a higher percentage of germination than seed harvested after ripening. It has been suggested (49) that seeds sometimes grow better just before ripening due to the presence of enzymes and protein-splitting products which decrease in amount with ripening as proteins are stored. However, if coat restrictions be concerned in the delay of after-ripening, it is possible that ripening tends to increase the impermeability of this coat. It was also found that improved germination could be secured by complete separation of the embryo from the endosperm. Other workers have successfully grown seeds thus mutilated (30, 65). The results secured with *A. fatua* may be illustrated by the instances given in table V.

TABLE V

Time	No. tested	Embryos alone per cent	Check per cent
December 1911...............	100	87	35
December 1912...............	100	87	48

4. RESPIRATORY RATIO.—In considering the effect of breaking the seed coat and its relationship to germination, the question arose as to whether if the seed coat acted as a restriction to oxygen entry, it might not be possible that after-ripening consists in a developing ability of the seed for anaerobic respiration. This was found not

to be the case. The results obtained, however, were interesting in another way. In making these tests, 25 seeds with like control were soaked over night in distilled water in the ice chest, and then placed in inverted test tubes over mercury and kept in the dark at a quite evenly registered temperature of about 21° C. Seeds were allowed to remain about four hours, a period during which the oxygen content of the tube was found not to fall below 16 per cent. At the end of the period the gas was drawn off and analyzed for carbon dioxide and oxygen with a Bonnier and Mangin gas-apparatus in the customary manner. From this data the respiratory ratio $\frac{CO_2}{O_2}$ was computed. Tests were made for the seeds intact, seared, and intact but in 93 per cent oxygen. Anaerobic respiration apparently did not increase in the three months (January to March 1913) during which these tests were being carried on. The point to these results, however, may be noted on taking the average of many determinations made under the three conditions given in table VI.

TABLE VI

	Ratio average
Seeds intact in pure air.............	0.800
Seeds seared......................	0.649
Seeds in 93 per cent oxygen........	0.557

It is interesting to note the decrease of the ratio as the seeds are seared, and an even greater decrease as they are subjected to increased oxygen concentrations. The results do not seem to be out of harmony with the conception of the seed coat acting as a restriction to oxygen entry.

5. GERMINATIVE TESTS UNDER VARYING CONDITIONS OF OXYGEN.—If the coats should thus tend to exclude oxygen, we might suppose that seared seeds would be able to germinate in an atmosphere of reduced oxygen content as well as intact seeds in air; while intact seeds, it might be supposed, would be benefited in germination percentage if placed in an atmosphere of increased oxygen content. To test out these hypotheses, the seeds were placed on moist absorbent cotton in dishes on tripods, and covered by an

inverted battery jar set in a water seal so as to leave the seeds 2.25
liters of gas, which was introduced by displacement. All was
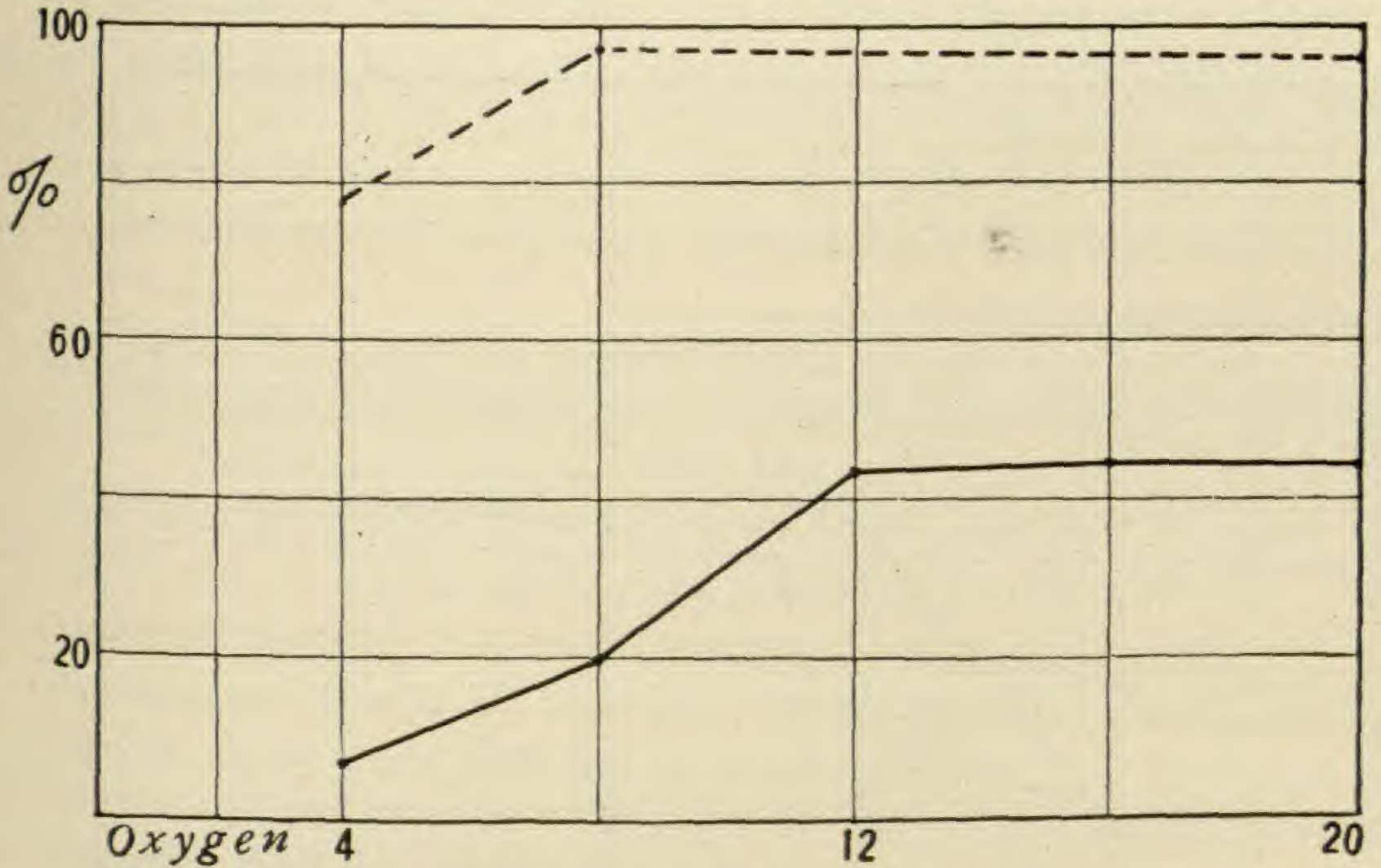

FIG. 3.—Percentage of germination of wild oats in reduced oxygen concentrations;
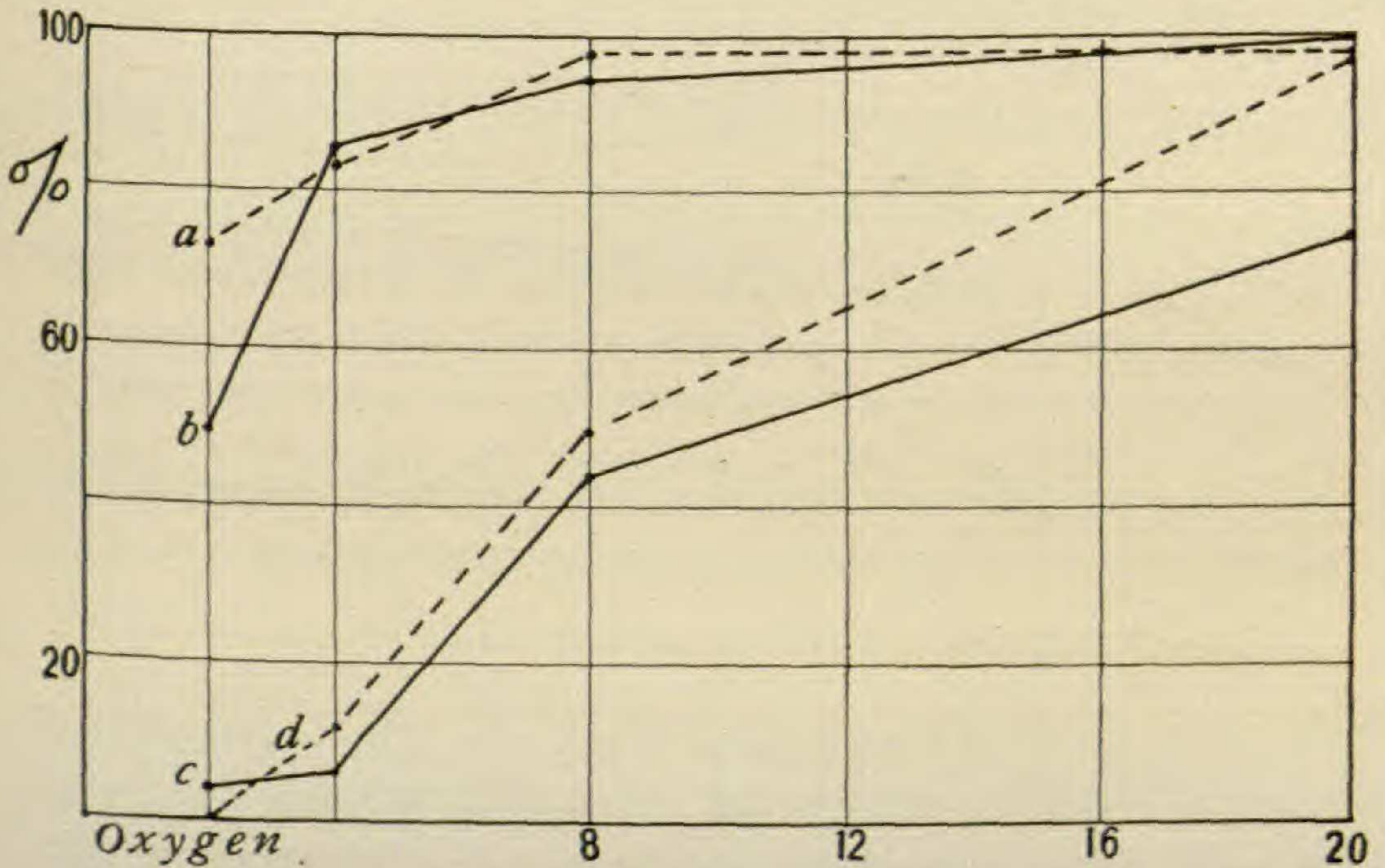intact seeds, solid line; seared seeds, broken line.

FIG. 4.—Comparison of germination percentages of tame and wild oats in reduced
oxygen concentrations; intact seed, solid line; seared seed, broken line; *Avena
saliva,* curves *a* and *b*; *Avena fatua, c* and *d*.

placed in a dark room at about 20° C. For oxygen content less than the approximate 20 per cent of air, hydrogen was used as dilutant; while for increased oxygen content, the gas was added
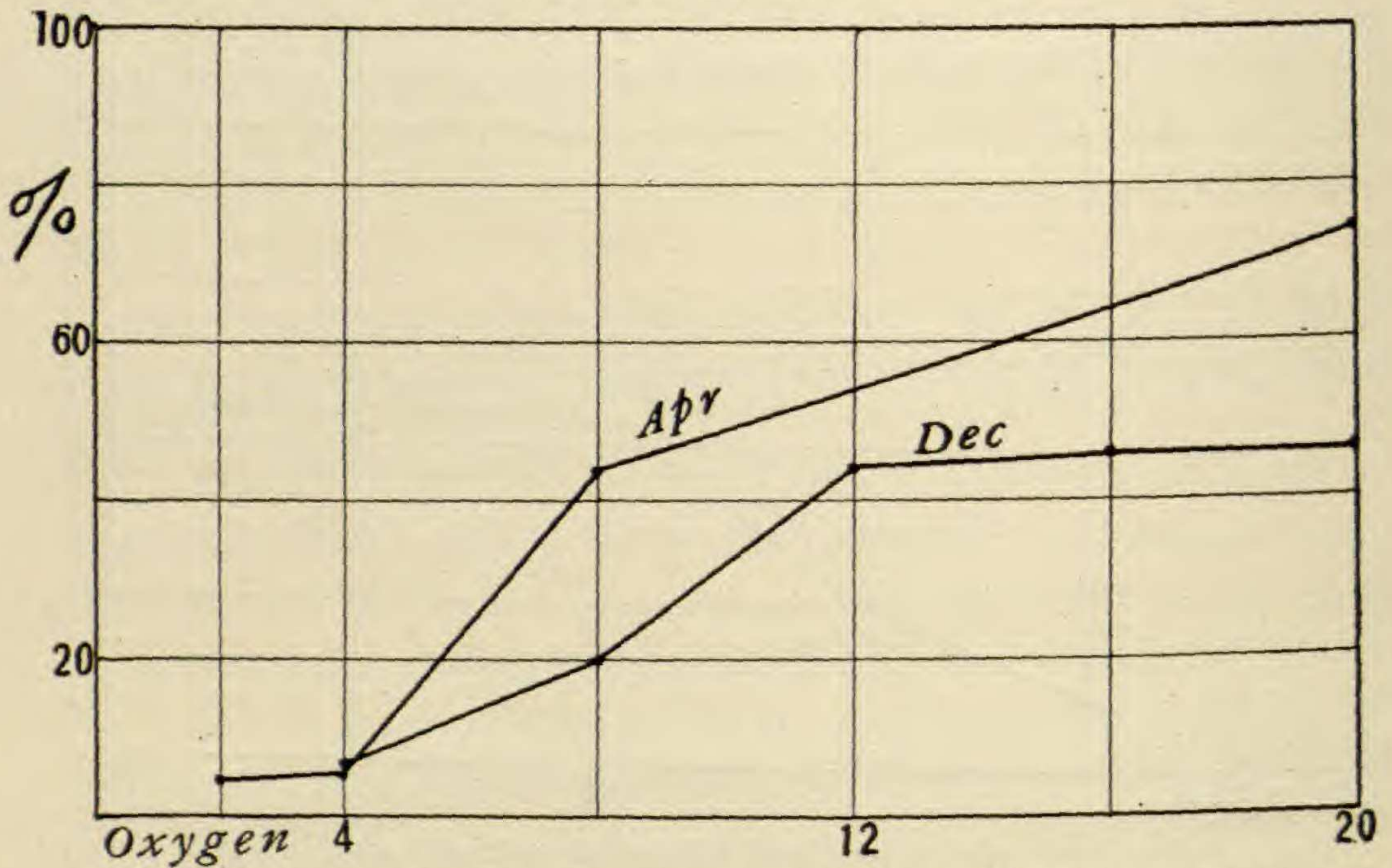

FIG. 5.—Comparison of germination percentages of wild oats in reduced oxygen concentrations in autumn and spring.
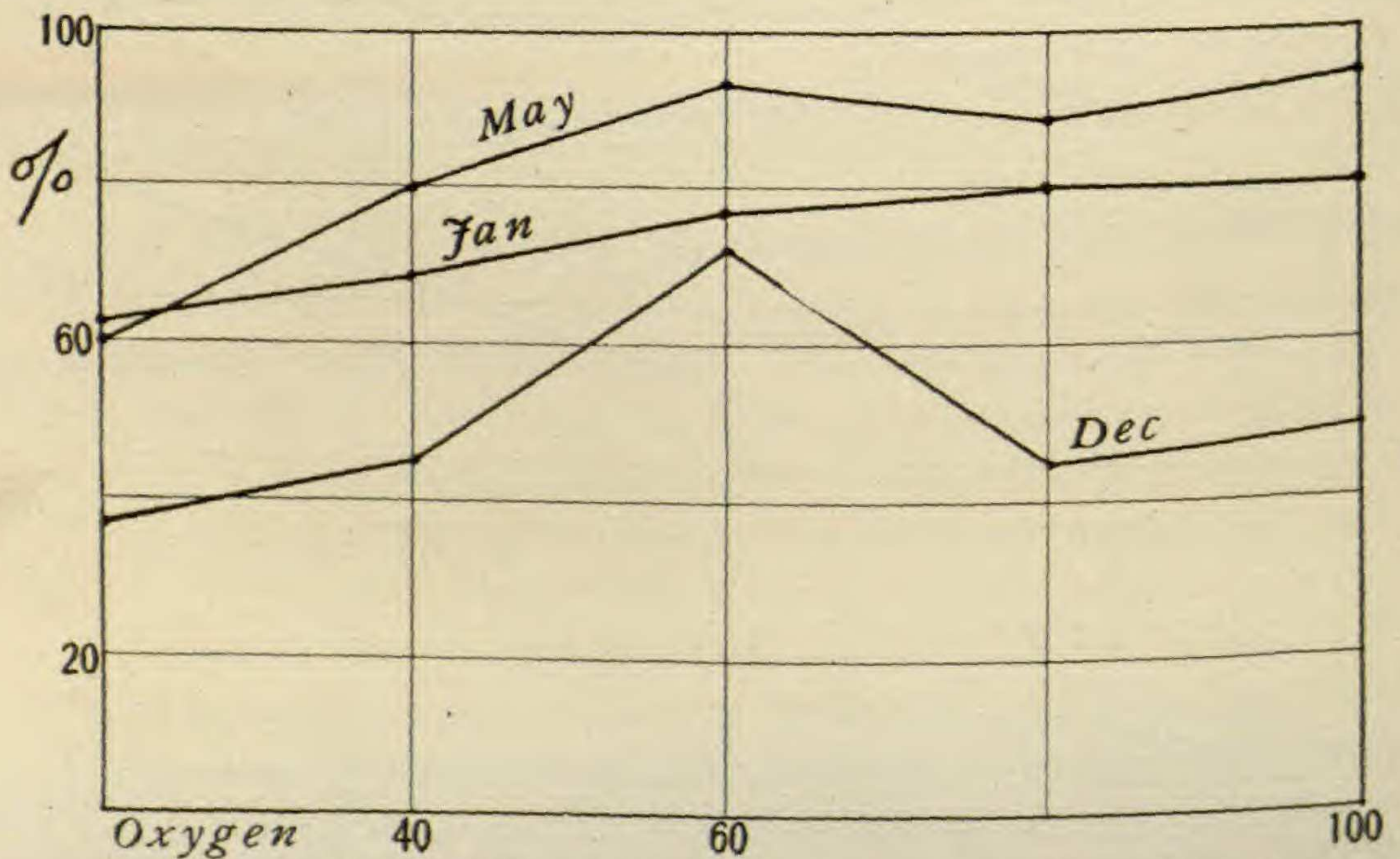

FIG. 6.—Comparison of germination percentages of wild oats through after-ripening period in increased concentrations of oxygen.

directly. The gas used was generated electrolytically by the American Oxhydric Company of Milwaukee, and showed high percentage of purity on analysis. Considering the air check as 20 per cent oxygen, tests were made at the further percentages of 2, 4, 8, 16, 40, 60, 80, and approximately pure oxygen. Comparing the effect of reduced oxygen content on the germination of both intact and seared seeds of *A. fatua*, greater ability is noticed for the seared seeds to grow with good percentages in concentrations of one-fifth the normal oxygen during December (fig. 3).

For April and May, if we compare the sound and seared seeds of *A. fatua* and *A. sativa* as shown in fig. 4, it is evident that searing increases the germination of both at all percentages of oxygen, the difference caused by searing being greater for the wild oat. The tame oat shows a high germination rate even in very low concentrations of oxygen. If we follow the intact wild oat through the winter, there is a noticeable rise in the ability of the seed to grow at lowered oxygen concentrations (fig. 5). If, on the other hand, increased concentrations of oxygen be tried upon *A. fatua* through the after-ripening period, there is noticeable a larger percentage of germination with increased oxygen concentrations up to about 60 per cent, beyond which point the seedlings tend to become stunted. The rise in viability from December to May is also noticeable (fig. 6).

6. MEASUREMENTS OF OXYGEN ABSORPTION.—The above results with variations in the oxygen concentration and in the season of the year may indicate either that as the season progresses there is a decrease in the embryo requirements for oxygen necessary to secure germination, or that there are modifications taking place in the seed by which the entry of oxygen may be more easily accomplished. To determine the nature of the rates of absolute oxygen absorption under these varying conditions, and as compared with *A. sativa*, a modification of the eudiometer, as devised by CROCKER, was employed (fig. 7). Seven seeds were used in each chamber. They were weighed to 0.1 mg., and moisture content determined in other seeds from the same lot, from which data the dry weight of the seeds used was computed. Before setting up the apparatus, the seeds were soaked over night in distilled water in the ice chest.

The seeds tested were suspended from platinum baskets at *A*, being laid on moistened neutral asbestos fiber, the non-organic substance being used to prevent drying out during the test, and at the same time to avoid danger of respiratory changes. Under the baskets were placed small vessels, each containing 1 cc. of 4 per cent potassium hydroxide solution to absorb any carbon dioxide evolved during the period. Test tube *D* was filled with mercury and could be raised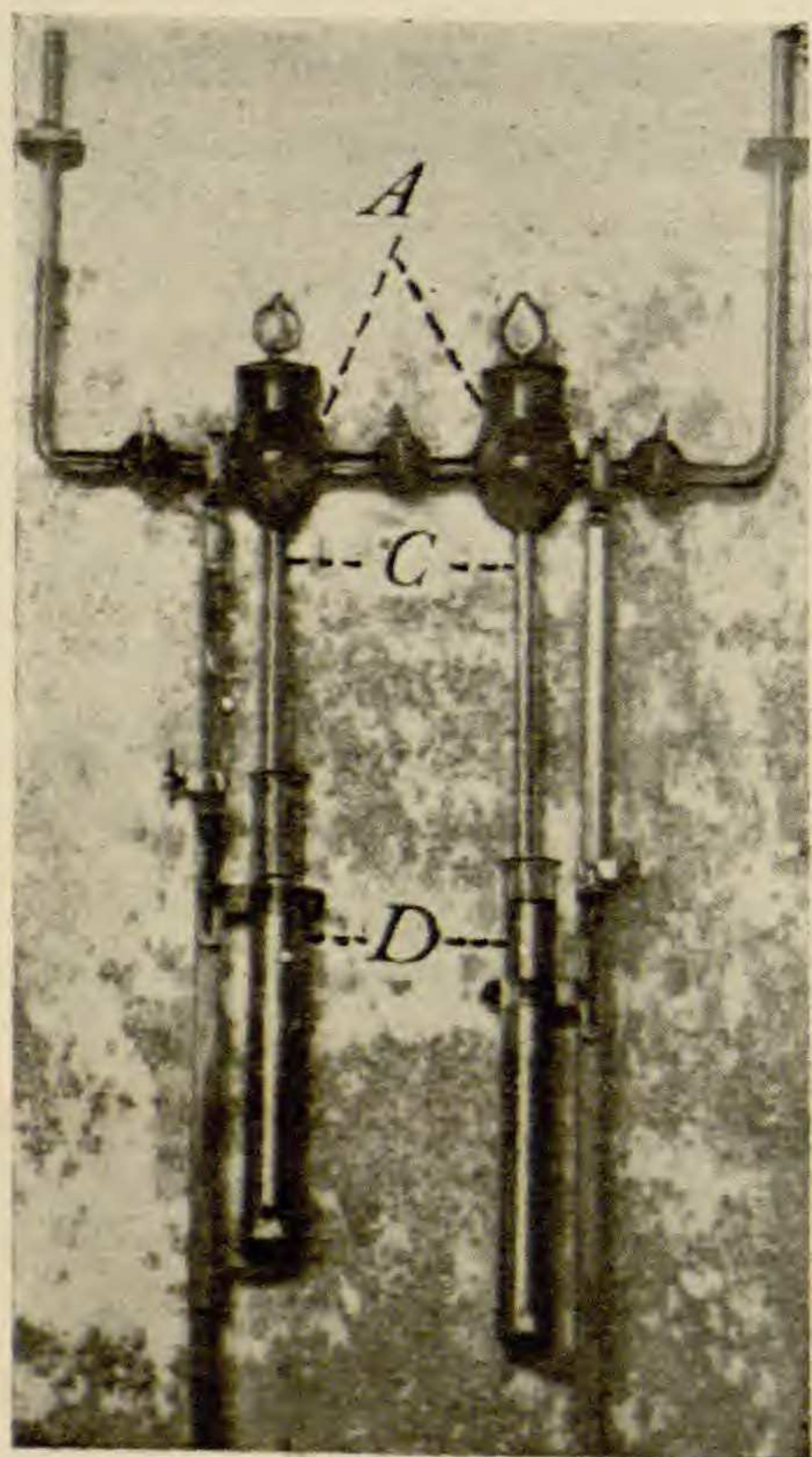 and lowered to balance the mercury column in *C* with that in the test tube. Tube *C* was 15 cm. long and had a volume of 2 cc., the graduations for which were made so that readings could be made to 0.01 cc. When the rate of oxygen intake from the air was to be measured, the gas in the apparatus was previously displaced for several minutes with a current of air forced through 40 per cent potassium hydroxide solution to remove any traces of carbon dioxide. Other gases tested were passed through the alkali and apparatus in like manner. During the course of taking the readings, the apparatus was submerged in a Freas constant temperature water bath electrically regulated and accurate to about 0°.01 C. The volume

Fig. 7.—Respirometer

of the chambers was so selected that variations in temperature would introduce an error so small as to be negligible with the water bath used. Thus, for the right chamber a variation of 0°.1 C. would result in an error of 0.0056 cc., and for the left chamber of 0.0062 cc. Corrections for barometric and temperature variations were made; hence any rise of the mercury column as shown by the corrected readings indicated absolute absorption of oxygen by the seeds tested. From the basis of the dry weight of the seeds used the rate of oxygen absorption per gram per hour was com-

puted for the various periods tested.   The two chambers served to check all readings.   It was found that quite consistent rates were obtained.   The rates of oxygen absorption were measured with the bath at the temperature of 21° C.

Before considering the data derived from the respirometer readings, it must be borne in mind that one of the objects sought is to gain a clearer idea of any changes which may occur in the embryo during the after-ripening period.   The gas absorption measurements are for the whole seed, of which the embryo forms, by weight, a small part. Hence any variation in the corrected reading for the whole seed might indicate a far greater comparative variation for the embryo.   STOWARD (63) has compared the respiration of the separated endosperm and embryo of barley. He finds that per gram of fresh weight, the respiration of the embryo alone is 17 times that of the endosperm, but as the endosperm weighs about 17 times as much as the embryo, these parts probably about halve the respiration of the intact seed.   To reason from the respiratory behavior of the separated units to the nature of the respiration of the intact seed seems sufficiently uncertain to justify at least
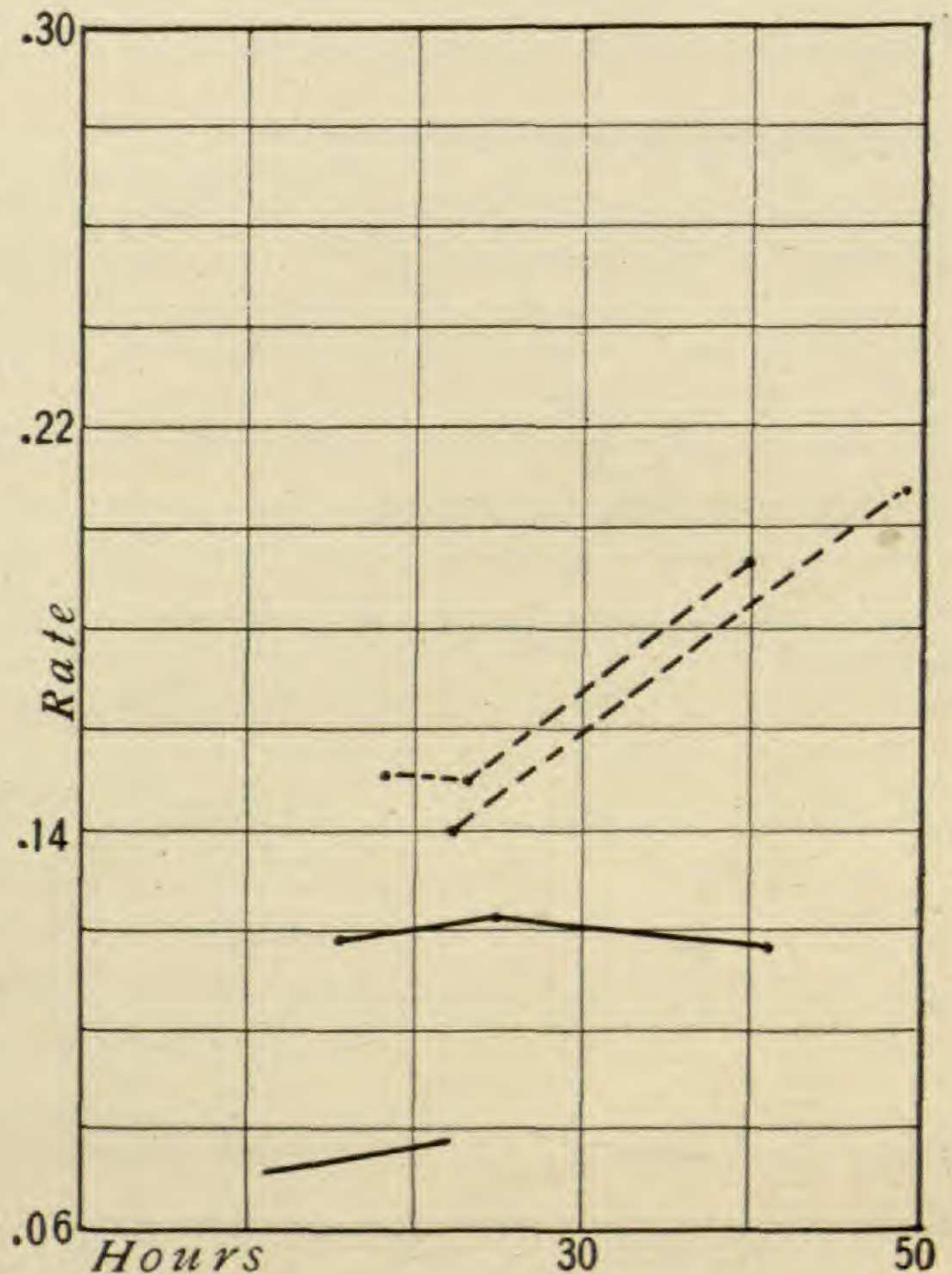

FIG. 8.—Rates of oxygen absorption for wild oats during December and early January in terms of cc. per hour per gm. dry weight; rate for intact seeds, solid line; for seared seeds, broken line.

calling attention to the fact of the combined participation of the embryo and endosperm in the data below.

From a large number of determinations the following will give an idea of the results. Each curve represents the average of the rates derived from both c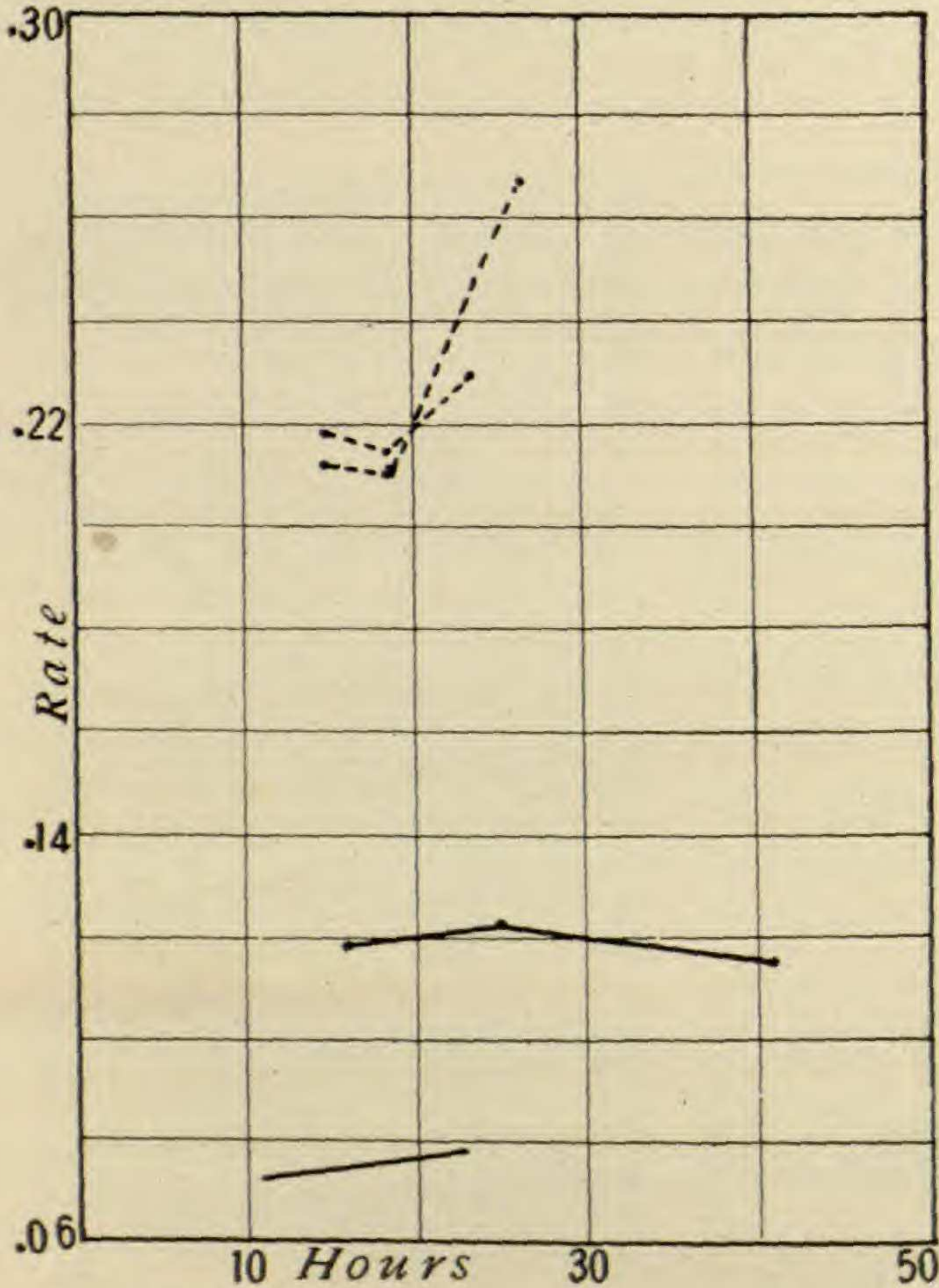hambers for the periods tested. In fig. 8, curves are shown indicating the absorption rates for intact and seared seeds. These determinations were made during December and early January, when the germination was averaging about 60 per cent for intact seeds. It is evident that the absorption rate is raised noticeably by searing. In most of the curves, a steady tendency is noted for the rate of absorption to rise with the length of the period tested. In many cases the high absorption noted beyond the 25–30 hour periods is associated with the breaking of the seed coats in germination. Earlier increases in rate, however, must not be ascribed to this cause.

FIG. 9.—Rates of oxygen absorption for wild oats for December and early January in terms of cc. per hour per gm. dry weight; rate for intact seeds, solid line; for seeds tested in 93 per cent oxygen, broken line.

ever, must not be ascribed to this cause. If now for this same period comparison be made of the absorption rates in the air and in an atmosphere of 93 per cent oxygen, the difference between the two conditions is marked (fig. 9). Further tests in 79 per cent oxygen revealed rates midway between those found for air and for 93 per cent oxygen; while absorption in 7 per cent oxygen

showed much lower rates for the same period than were obtained in air.

Oxygen absorption tests of freshly harvested wild oats were made during July 1913 at temperatures of 16°.2 C. and 26°.2 C. It was thought possible that the failure or success of the VAN'T HOFF temperature law of chemical reaction to hold with the fresh seed might, in connection with the other data, throw some light on the power of the seed coat to exclude oxygen. Summarizing a number of readings, it was found that

$$\frac{\text{the rate at } 26°.2 \text{ C.}}{\text{the rate at } 16°.2 \text{ C.}} = \frac{0.160}{0.067} = 2.38.$$

This is quite what might be expected if the coat offered no restriction to gaseous penetration, and appears at first to conflict with the data derived on this point in other tests. However, we cannot as yet say what effect such a temperature change may exert on the permeability of the coat, which is a non-living structure. It is a well known fact that the solubility of oxygen in water decreases with a rise in temperature. Thus, the absorption at 35° C. is 56.9 per cent of that occurring at 5° C. GASSNER in a recent article (28), as reviewed by LEHMANN (48), believes that the beneficial results obtained in germination of *Chloris ciliata* through the use of low temperatures may be due to the greater absorption of oxygen at these temperatures. He employed temperatures varying from 5° C. to 34° C. LEPESCHKIN (50) and others, however, have pointed out the fact that the permeability of living protoplasm to gases increases with rising temperature. It is thus quite possible that the problem of oxygen absorption by the grasses may be complicated by the opposite influence of high temperature on the solubility of oxygen in the water in the seed, and on the permeability of the seed coat itself. Conclusions on these tests must hence be delayed pending further investigation of the effect of varying temperatures on the permeability of non-living membranes to gases.

As it was noted that in every germination test a large number of seeds laid dormant, yet if forced by searing would promptly germinate, the experiment was tried of testing the oxygen-absorption

rate with seeds selected after lying dormant five days under the usual germinative conditions. The results showed a much lower absorption rate, both in air and in 93 per cent oxygen, than was the case in tests made with unselected seed. Furthermore, the difference between the rates in air 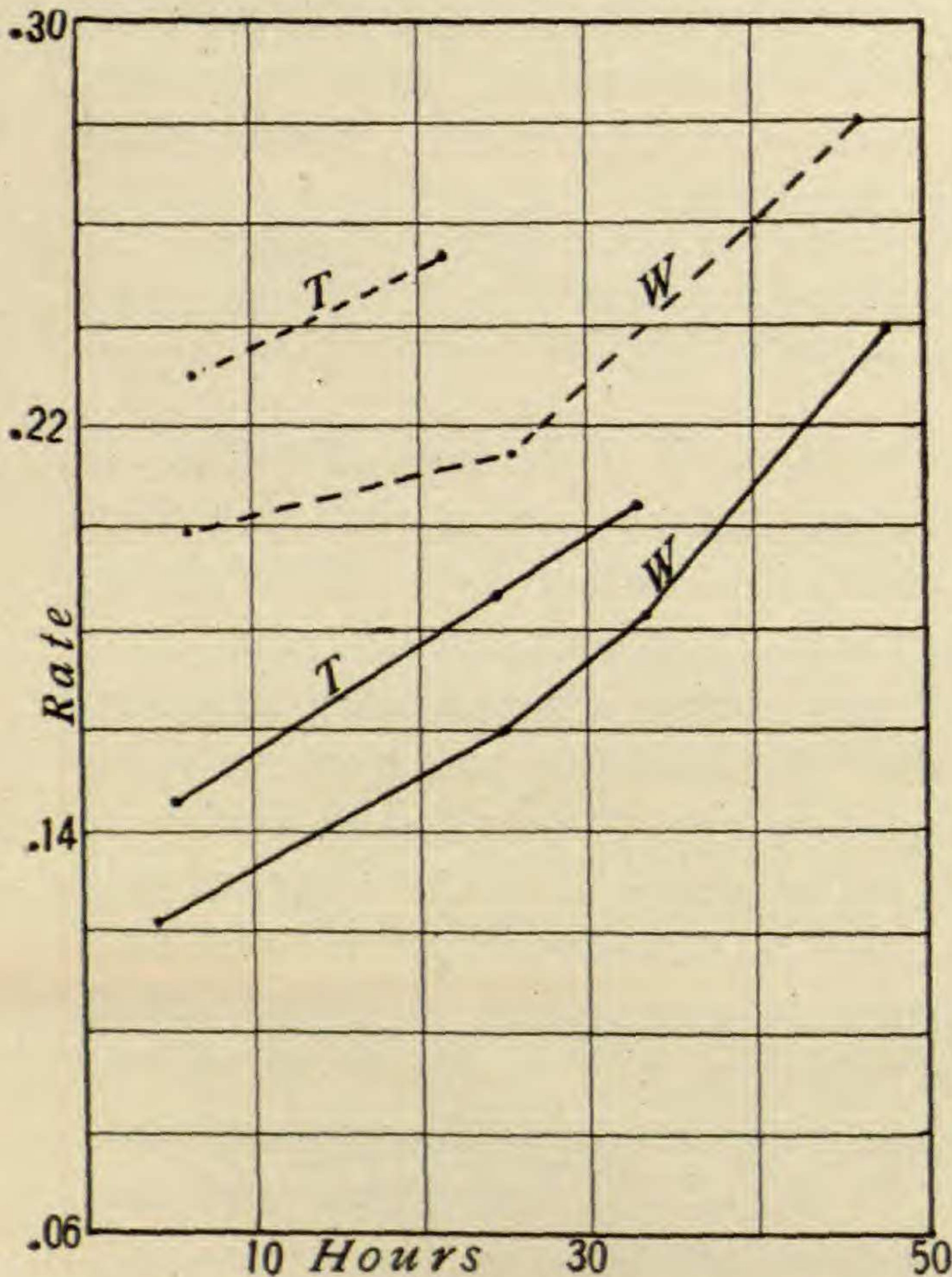and in higher concentrations of oxygen was not so marked. It is realized, of course, that the "selected seed" was not in behavior to be strictly compared with seed treated in the usual manner, as they were subjected to a temperature of 21° C., in the period that germination was attempted before the respirometer test.

The oxygen absorption seemed to be practically the same for seeds seared dry one month before testing, and those seared after one night of soaking in the ice box, just previous to placing in the respirometer. The similarity in germinative behavior of the seeds treated in these two ways has been noted above. Other workers (6, 58, 62) have studied the influence of wounding on respiration as measured by the resultant carbon dioxide releasal. JUNITZKEY (40) holds that oxygen absorption and carbon dioxide releasal may be phenomena independent of each other. In any case, we are concerned here only with the direct rate of oxygen absorption by the seed as bearing on the problem of germination. For this

FIG. 10.—Comparative rates of oxygen absorption for tame and wild oats in terms of cc. per hour, per gm. dry weight; *Avena sativa* indicated by *T*, and *Avena fatua* by *W*; rates for intact seeds, solid line; for seared seeds, broken line.

reason it is of interest to note that the temporary "wound effects" noted for other tissues with carbon dioxide as a basis of decision, do not hold for the oxygen intake of *Avena fatua*.

As the early germinative delays of *A. sativa* are so much less pronounced than is the case for *A. fatua*, it is of interest to compare the oxygen-absorption rates for the two (fig. 10). The tame oat shows a higher rate throughout. It was impossible to carry out these tests (summarized in fig. 10) until April, when the comparative difference in germinative delay between the tame and the wild oat is much less than in the preceding autumn. This makes it seem probable that when fresh seed may again be obtainable, the differences in rate found above, though quite marked, may be even more conspicuous.

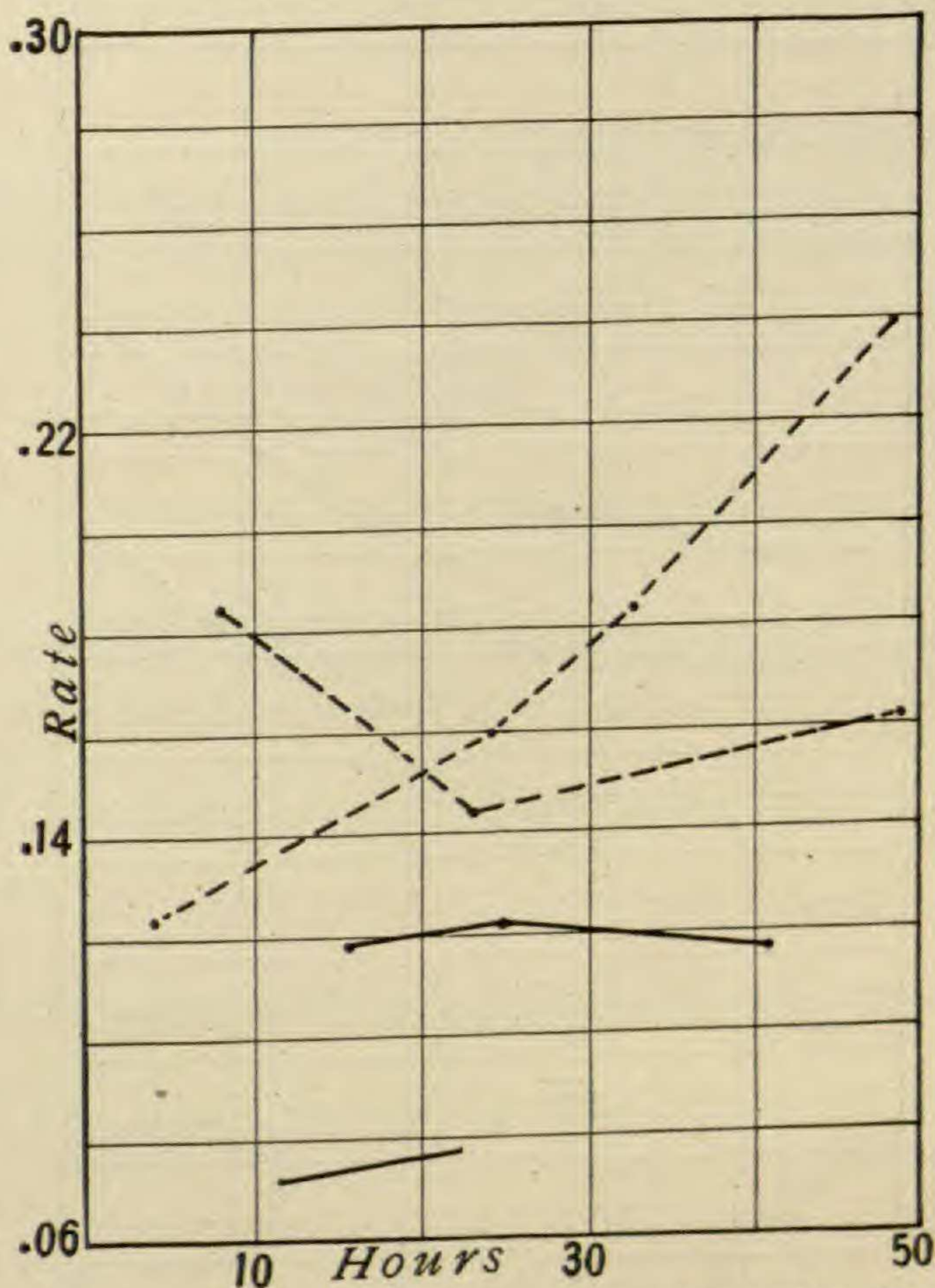In figs. 11–13 are shown respectively

FIG. 11.—Rates of oxygen absorption for intact wild oats before and subsequent to after-ripening in terms of cc. per hour per gm. dry weight; rates in winter, solid line; in spring, broken line.

the effect of after-ripening on the rate of oxygen intake by intact seeds, seared seeds, and seeds run in an atmosphere of over 90 per cent oxygen. The temperatures employed were identical in the 24 tests here summarized. In each case the winter rate is indicated by the unbroken lines, and the spring rate by broken lines. A consistent increase in the rate of oxygen absorption in

higher concentrations is shown. This increase is least noticeable in the case of the high percentage of oxygen tests. It is suggested that if coat restrictions are concerned, and if their effects are overcome by high concentrations of oxygen, the four curves would tend to come together, as is found to be the case.

Summarizing the respiration tests, it would seem that associated with increased germinative rates accompanying afterripening there is an increased ability of the seed to take up oxygen, providing always that the conditions of germination be the same; and, further, that wounding and subjecting to increased oxygen concentrations actually increases the oxygen intake. Nevertheless, the process of after-ripening may not consist primarily in an increased ability of the embryo to take up oxygen. If the unafter-ripened seeds be freed from limitations to absorption, as was ac-
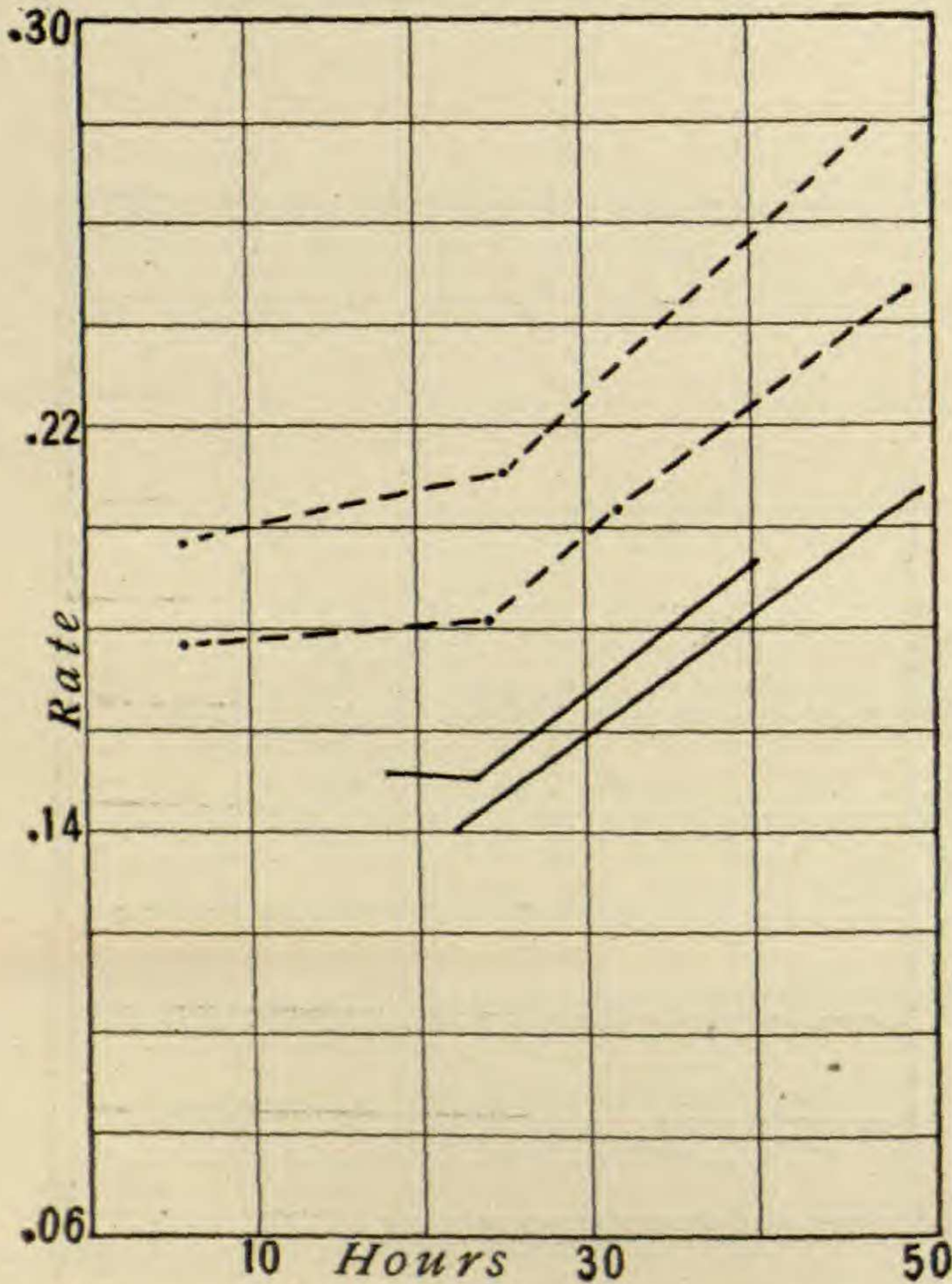

FIG. 12.—Rates of oxygen absorption for seared wild oats before and subsequent to after-ripening in terms of cc. per hour per gm. dry weight; rates in winter, solid line; in spring, broken line.

complished in the experiments summarized in fig. 12, it is apparent that their oxygen-absorption rate closely approximates that of the seeds which have thoroughly after-ripened. Thus the changes, whether they be seed coat or embryonal, which we ordinarily term after-ripening, and which are exhibited by increased rate of oxygen intake, may be immediately attained by artificially overcoming

the limitations to oxygen entry, if rate of absorption be taken as a basis of judgment.

7. DETERMINATION OF EMBRYO ACIDITY.—Investigation has been begun as to variations in the acidity of the embryo in after-ripening, and comparisons have been made with the embryos of A. sativa. To test this condition, the seeds were soaked over night in the ice box, and then subjected to identical germinative conditions for about 18 hours. The embryos were then removed, accurately weighed, carefully ground, and immediately titrated with N/20 alkali in the presence of phenolphthalein. Water content of the embryos was determined on other parallel samples, from which data the dry weight of the titrated embryos could be computed. Figuring then the number of cc. of N/20 alkali necessary

FIG. 13.—Rates of oxygen absorption for wild oats in high concentrations of oxygen before and subsequent to after-ripening in terms of cc. per hour per gm. dry weight; rates in winter, solid line; in spring, broken line.

to neutralize the acidity of the equivalent of one gram dry weight of the embryos, the results given in table VII were obtained.

It will be noticed that the 1912 samples grown at Chicago were tested early and were less acid than corresponding year old samples of both the tame and of the wild. Further, the acidity is less in A. fatua seeds one year old than in A. sativa just harvested.
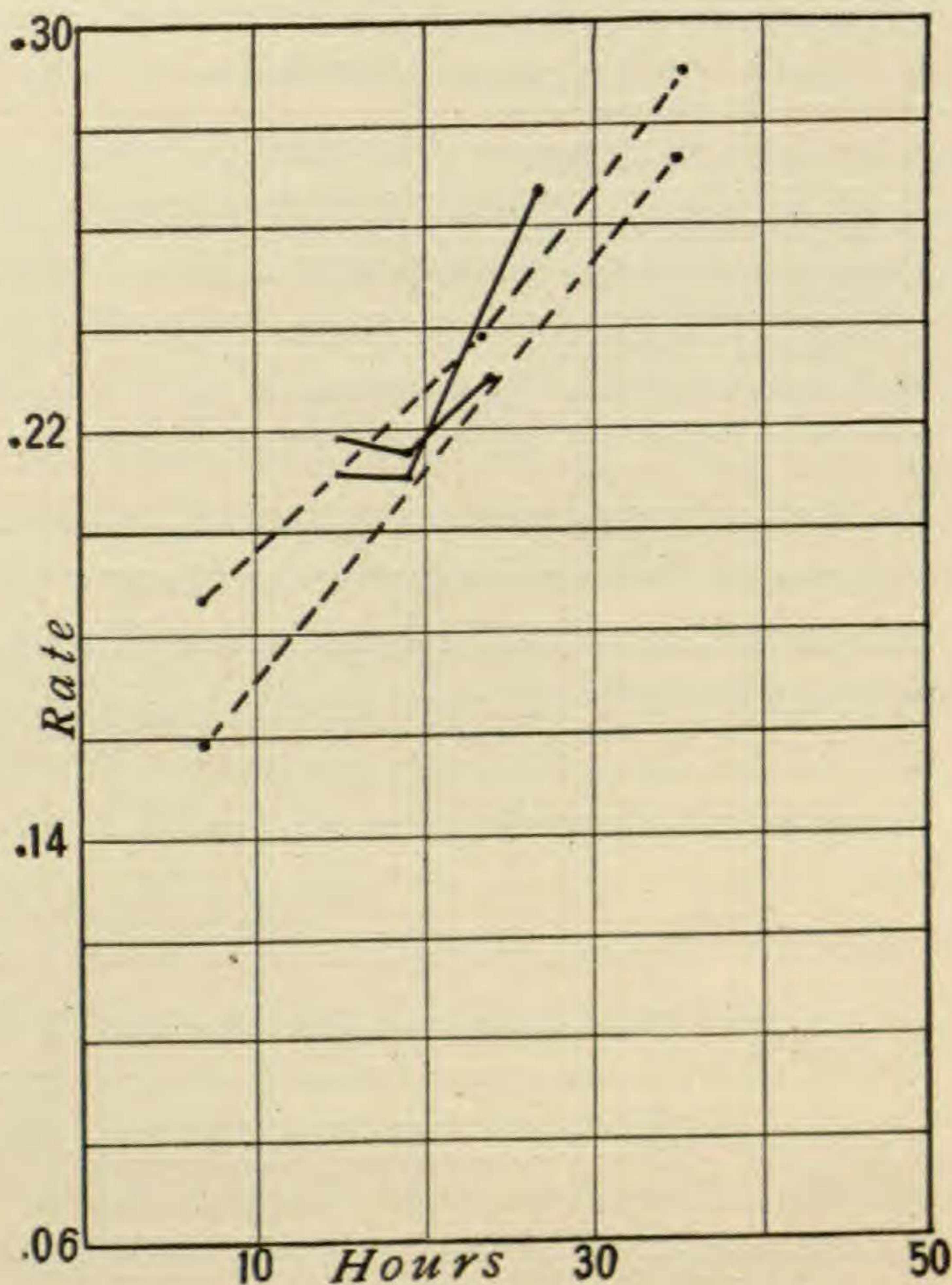
Comparing the degree of acidity of the various samples with the moisture contained in the respective embryos, the relations appearing in table VIII are found.

TABLE VII

EMBRYO ACIDITY COMPARISONS OF AVENA FATUA AND A. SATIVA

TESTED AUGUST 1912		N/20 ALKALI FOR 1 GM. DRY WEIGHT
Kind	Season grown	
Tame (Swedish select)...............	1911	3.70
Tame (Swedish select) tested fresh.......	1912	2.51
Tame (Kherson)....................	1911	3.37
Wild (Indian Head).................	1911	2.37
Wild (grown Chicago) tested fresh.......	1912	1.87

It is seen that there is a general tendency for the water-holding power and the acidity to rise contemporaneously. This situation was noted by Miss ECKERSON for *Crataegus*. It is possible that such embryonic changes in *A. fatua* may be causally related to alterations in inclosing structures. Further investigation of the chemistry of the embryo is planned.

TABLE VIII

N/20 alkali to titrate 1 gm. dry weight	Per cent water in embryos
1.87..............................	50.2
2.37..............................	56.0
2.51..............................	54.3
3.37..............................	71.0
3.70..............................	68.3

8. CONCLUSIONS.—The combined results, so far noted, namely, that germination can be increased by various coat-breaking methods; that germination may at all stages be improved by increased oxygen; that when wounded or subjected to increased concentrations of oxygen there is an absolute increase in the rate of oxygen absorption; all seem to point to the conclusion that oxygen supply is for the freshly harvested wild oat the limiting factor to germination, with the probability that coat restrictions to oxygen entry play a rôle. The question still is open as to the nature of the physiological processes for which oxygen is thus essential. GRÜSS (31) believes that in the case of *Phaseolus* the abundant

enzyme content in the cells neighboring a wound occurs as a result of the action of oxygen on the reserve proteins. LEHMANN (49) believes germination stimuli are effective through their influence on protein hydrolysis. The work of Miss ECKERSON and of GREEN suggests the possibility that the development of acidity leads to the liberation of enzymes. What relationship, if any, there may be between acidity, oxygen, enzymes, and germinating power in *A. fatua* is worthy of further investigation.

The character of the changes in the seed of *A. fatua* with after-ripening remains to be discovered.

Oxygen being considered the limiting factor to germination for the freshly harvested seed, it is possible to consider that the embryo in the course of after-ripening either decreases in its demands for oxygen, whereby the seeds become able to grow in gases poor in oxygen; or we may suppose that there is no decrease in oxygen demands, but rather an increased permeability of the coat to oxygen. The fact that after-ripened seeds can grow better than fresh seeds in chambers poor in oxygen, although the former regularly absorb oxygen at a more rapid rate in respiration under normal conditions, together with the results (as shown graphically in fig. 13) that fresh and after-ripened seeds in the presence of high percentages of oxygen absorb at similar rates, seem to favor, but not to prove, the general idea that the coat exclusion to oxygen becomes less complete as the seed after-ripens. What mechanism is released by the greater oxygen supplied either artificially through breaking the coat, or submitting the seed to high percentages of oxygen, or under natural conditions through a slowly developed increase in the coat's permeability to oxygen, is as yet an open question. If further investigation upholds the data given above, showing an increased acidity of the embryo with after-ripening, we must recognize the fact that in *A. fatua* after-ripening involves, in addition to physical changes of the coat, accompanying chemical alterations of the embryo itself.

IV. Summary

1. The germination of *A. fatua* has been found less delayed with the shell coats removed from the seed. However, with the shell coats removed, there exist after harvest germinative delays

which disappear with subsequent weeks. Hence the after-ripening of the seed occurs independent of the shell coats.

2. The after-ripening occurs along with the drying of the seed, but independent of the water content, as air-dried seed soon after harvest yields lower germinative percentages than seeds of similar moisture content the succeeding spring.

3. The germination seems unaffected by light.

4. Exclusion of water by the true seed coat does not seem to explain after-ripening.

5. The delay in germination is occasioned by restriction in the supply of oxygen, which thus acts as a limiting factor to germination. The seed coat is probably an obstruction to oxygen entry. This general situation seems pointed to by the combined results obtained by breaking and searing the seed coat; by removal of the embryo; by germinative percentages obtained in varying concentrations of oxygen, both below and above the normal of the air; by direct measurement of the rate of oxygen intake with intact and seared seeds, and with seeds in varying concentrations of oxygen.

6. The exact nature of the changes in the seed which constitute after-ripening cannot be stated positively. However, the data obtained seem to point to an increased permeability of the seed coat to oxygen, together with a rise in the embryo acid content, which is accompanined by increased water-absorbing power of the embryo.

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